

ABSTRACT

A cell-free assay system, which reconstitutes components of the phosphatidylinositol 3-kinase-mediated insulin signaling pathway including phosphatidylinositol phosphate dependent kinase-2 ("PDK2"). Alternatively, a *in vitro* method for phosphorylating a protein kinase B on Serine 473 or Serine 474. The invention relates generally to an *in vitro* method of phosphorylating a protein kinase B ("PKB" or "Akt"), to an *in vitro* method of assessing insulin action, and to an *in vitro* method of identifying an agent or process that modulates insulin signaling or any cellular activity regulated or influenced by PKB, including cell growth, mitosis, apoptosis, fuel metabolism, and oncogenic transformation. Such an agent or process may be useful in treating insulin resistance, diabetes, obesity, cancer, and a number of other diseases.